REMARKS

I. The Invention

The present invention relates to a new method for measuring the amount of cholesterol present in the form of different lipoproteins, such as low density lipoprotein (LDL) and high density lipoprotein (HDL), as well as the total amount of cholesterol in a sample. In this method, a complex comprising a first lipoprotein fraction (*e.g.*, LDL) is formed and therefore renders the cholesterol associated with this lipoprotein fraction unavailable for measuring. The amount of cholesterol not involved in the complex (*i.e.*, cholesterol associated with non-LDL) is then measured. Subsequently, the complex is dissolved, making the cholesterol associated with the first lipoprotein fraction available for measuring, and the amount of total cholesterol in the sample is then measured. Through subtraction, the amount of cholesterol associated with the first lipoprotein fraction can be determined. The claimed method provides a novel means for measuring the amount of cholesterol associated with different lipoproteins in the same test tube.

II. Status of the Claims

Claims 1-29 were originally filed. In response to a restriction requirement, claims 1-21 were elected. Upon entry of the present amendment, claims 22-29 are canceled without prejudice to future revival. Claim 1 is amended to recite "determining the amount of cholesterol in the first lipoprotein fraction present in the sample by subtracting the first cholesterol value from the total amount of cholesterol present in the sample," support for which can be found throughout the specification, *e.g.*, in the Abstract. Claims 1-3 are also amended to recite "wherein" in place of "with the proviso that" and "further provided that." Claims 5 and 9 are amended to add the missing period at the end of the claims. No new matter is added.

III. Claim Rejections

A. 35 U.S.C. §112, Second Paragraph

Claims 1-6 and 8-21 were rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness, as the Examiner contended that the claims failed to "particularly point out

and distinctly claim the subject matter which applicant regards as the invention." Applicants respectfully traverse the rejections, particularly in light of the present amendment.

The Examiner rejected claims 1, 4-6, 17, and 19 for reciting the word "fraction." The Examiner alleged that the word is indefinite because "it is not clear whether/how a sample is fractioned or fractionated," or how a fraction is determined, or "whether/how said fractions are created, identified, isolated, separated, distinguishable, or ... physically divided from the rest of the sample." Applicants respectfully disagree.

The method of the present invention provides a novel, sequential approach for determination of the amount of cholesterol present in different lipoprotein species in a sample and the total amount of cholesterol in the sample. As the first step of this method, a complex-forming agent is added to a sample to form a complex with a first lipoprotein fraction, such as LDL. The complex traps the cholesterol associated with the first lipoprotein fraction and renders the cholesterol unavailable to be measured. Once the complex is formed, remaining cholesterol in the sample is measured. Subsequently, the complex is disrupted, releasing the cholesterol associated with the first lipoprotein fraction for measuring, and total cholesterol in the sample is thus determined. According to this method, there is no need for physically separating the different lipoprotein fractions in a sample; in fact, the make-up of a particular lipoprotein fraction is determined based on the species of lipoprotein(s) that can interact with a given complex-forming agent, such as an anti-apoB antibody, to form a cholesterol-sequestering complex. Only upon the formation of such a complex, a lipoprotein fraction is created or distinguished from the reminder of the sample. *See, e.g.*, page 7, lines 21-32. This fraction is, however, never physically separated or isolated from the reminder of the sample. *See, e.g.*, page 8, lines 21-31. Thus, upon reading the specification, one of skill in the art would understand without any ambiguity how a lipoprotein fraction is determined or created and that there is no physical isolation, division, or separation necessary for distinguishing the different lipoprotein fractions.

Claim 1 was also held indefinite for the recitation of "a first cholesterol value." The Examiner questioned the purpose of this "first cholesterol value" for the determination of

total cholesterol level. The Examiner also asserted that step (d) in claim 1 is indefinite, stating that it is not clear how "the amount of cholesterol in the first and second lipoprotein fractions" can be determined.

The method of the present invention measures not only the total amount of cholesterol in a sample, more importantly, this method allows the measuring of the amount of cholesterol associated with different lipoproteins in the sample without the need to physically separate the lipoproteins. As explained above, the claimed method measures the amount of cholesterol associated with different lipoprotein fractions in a sample in a sequential manner: cholesterol present in a first lipoprotein fraction is made unavailable for measuring through complexing while remaining cholesterol in the sample is measured (*i.e.*, obtaining the "first cholesterol value"); the complex is then dissolved and all cholesterol in the sample is measured (*i.e.*, obtaining "the total amount of cholesterol present in the sample"). Thus, the "first cholesterol value" reflects the amount of cholesterol not associated with the first lipoprotein fraction. Although this value plays no direct role in the determination of total cholesterol, it is necessary for calculating the amount of cholesterol present in the first lipoprotein fraction, which is determined by subtracting the "first cholesterol value" from "the total amount of cholesterol present in the sample." Applicants thus submit that the "first cholesterol value" does serve an important purpose for the claimed method, and that it is clear how "the amount of cholesterol in the first and second lipoprotein fractions" is determined, especially after the present amendment.

The Examiner stated that language "with the proviso that" or "provided that" does not create a negative claim limitation. Applicants do not agree with this assertion, because this type of language is frequently found in issued claims. For instance, claim 3 of U.S. Patent No. 6,855,785 reads as follows:

> 3. A heat-curable composition comprising
>
> (I) at least one constituent whose molecule comprises on average at least one functional group selected from the group consisting of
>
> (A) functional groups containing at least one bond activated by means selected from the group consisting of heat, actinic radiation, and mixtures thereof,

> (B) reactive functional groups able to undergo thermal crosslinking reactions with reactive functional groups, complementary reactive functional groups, and mixtures thereof, and mixtures thereof, ***with the proviso that*** there are always groups (A) and (B) in the composition; and
>
> (II) a mixture of monomeric and oligomeric benzpinacol silyl ethers.

To expedite prosecution, however, the word "wherein" has been inserted into claims 1-3 to replace the language the Examiner objected to. This amendment merely substitutes wording with identical meanings and therefore does not affect the claim scope.

Claim 8 was rejected for lack of proper antecedent basis for reciting "said first lipoprotein." As amended, claim 8 now recites "said first lipoprotein fraction," which does have proper antecedent basis in claim 1.

The Examiner also questioned the language in claims 10 and 11, alleging that it is not clear how to measure the total amount of cholesterol when a portion of the cholesterol has apparently been consumed by reaction with cholesterol esterase, cholesterol oxidase, or cholesterol dehydrogenase.

Applicants respectfully direct the Examiner's attention to page 24, line 20, of the specification. In the paragraph bridging pages 24 and 25, it is stated that,

> Total cholesterol in serum is typically measured enzymatically using cholesterol esterase and cholesterol oxidase. Cholesterol esterase ("CE" or "CHE") converts cholesterol ester to free cholesterol. The free cholesterol is then oxidized by cholesterol oxidase ("CO"), which generates a molecule of hydrogen peroxide. The generation of hydrogen peroxide is then detected by the oxidation of various dyes by peroxidase. The oxidation of the reporter dye results in a change of its absorption spectrum, which is used to determine the concentration of total cholesterol.

In the next paragraph, beginning on page 25, line 5, the specification further describes that when cholesterol present in a lipoprotein fraction is blocked from reacting with cholesterol esterase and cholesterol oxidase, such as being trapped in an antibody-apoB complex, an absorbance reading is obtained when available cholesterol has been depleted. Subsequently, when the antibody-apoB complex is disrupted, more cholesterol becomes available to react with

cholesterol oxidase, an increase in absorbance is observed. Thus, it is clear that even though cholesterol does become depleted upon reacting with cholesterol oxidase, total cholesterol amount can be directly measured because of the accumulative nature of the absorbance-based method.

The withdrawal of the indefiniteness rejections under 35 U.S.C. §112, second paragraph, is respectfully requested.

B. 35 U.S.C. §102

Claims 1-6 and 8-21 were rejected under 35 U.S.C. §102(e) for alleged anticipation by Miki *et al.* (U.S. Patent No. 6,162,607). Applicants respectfully traverse the rejection.

To anticipate a pending claim, a prior art reference must provide, either expressly or implicitly, each and every limitation of the pending claim. MPEP §2131. The pending claims are directed to a method for sequentially determining amounts of cholesterol in different lipoprotein fractions present in a sample, as well as the total amount of cholesterol in the sample. This method comprises the following steps in this order:

(a) contacting a first lipoprotein fraction in the sample with a complex-forming agent to form a complex of said first lipoprotein fraction with the complex-forming agent, wherein the complex is not a substrate for cholesterol esterase;

(b) measuring the amount of cholesterol associated with a second lipoprotein fraction present in the sample, to obtain a first cholesterol value;

(c) dissociating the first lipoprotein fraction from the complex-forming agent; and,

(d) measuring the total amount of cholesterol present in the sample, and determining the amount of cholesterol in the first lipoprotein fraction present in the sample by subtracting the first cholesterol value from the total amount of cholesterol present in the sample,

thus determining the amount of cholesterol in the first and second lipoprotein fractions present in the sample.

In contrast, the Miki *et al.* references relates to a method for measuring the amount of a constituent (*e.g.*, cholesterol) in only one lipoprotein fraction, but not the amount of the constituent in the remaining fraction or the total amount of the constituent, in a sample. The Miki *et al.* method includes the following steps:

First, a mixture is made by adding into a sample an antibody, which binds specifically to the lipoproteins other that the specific species of lipoprotein to be measured.

Second, the optical absorbance (OD_1) of the mixture from the first step is obtained.

Third, a reagent capable of causing a reaction for measuring the constituent (*e.g.*, cholesterol) is added to the mixture from the first step, and the optical absorbance (OD_2) is obtained upon completion of the reaction. An exemplary reaction for this step is the reaction between cholesterol and cholesterol oxidase.

Fourth, the amount of constituent (*e.g.*, cholesterol) is determined based on the difference between OD_1 and OD_2.

See, e.g., column 2, lines 10-25; and column 6, lines 15-26, of the Miki *et al.* reference for more detailed description of the method. According to the Miki *et al.* method, when OD_1 is measured following the first and second steps, this absorbance value reflects merely the background reading of the antibody-lipoprotein complex, because no cholesterol-measuring reaction has taken place at that time. When OD_2 is obtained in the third step following the cholesterol-measuring reaction, the ***increase*** in absorbance reading reflects the amount of cholesterol present in the species of lipoprotein(s) not a part of the antibody-lipoprotein complex. The subtraction of OD_1 from OD_2 therefore only serves the purpose of eliminating background signal in the process of determining the amount of cholesterol of one fraction of lipoprotein(s) in a sample. The Miki *et al.* reference does not teach how to determine the amount cholesterol present in the remaining lipoprotein fraction or the total amount of cholesterol in the sample.

Since the limitations of the pending claims are not provided in the Miki *et al.* reference, this reference cannot anticipate the claims of the instant application. Applicants thus respectfully request the withdrawal of the anticipation rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Chuan Gao
Reg. No. 54,111

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
CG:cg
60422022 v1